Attachment of immunostimulant proteins to the surface of cancer cells using a pH triggered anchor

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We describe a new process for the linkage of immunostimulant or recognition molecules to the surface of cancer cells. We propose this approach as an alternative to transfection technologies for anti cancer immunotherapy. The diphtheria toxin transmembrane domain (T domain), a protein of 21 kDa, is water soluble at neutral pH and interacts with lipid membranes at mild acidic pH (pH 5). We have constructed a fusion protein, T-hIL-2, in which human interleukin 2 (hIL-2) was attached to the C-terminus of the T domain. The recombinant protein was expressed in E. coli. It was able to stimulate the proliferation of the IL-2 dependent murine spleen cell line CTLL-2, demonstrating functionality of the cytokine domain. It was also capable of interaction with lipid vesicles at acidic pH, demonstrating the functionality of the T domain. The fusion protein T-hIL-2 was able to bind to the surface of adherent and non adherent cells following incubation at pH 5, but not at pH 7.4. This was demonstrated by immunoenzymatic detection and fluorescence microscopy. Maximum binding occurred within an hour and the proteins remained on the cell surface for more than 24 hours without much decrease. Cell viability was mildly affected, if not, by the treatment. We have shown that murine lymphoma cells treated with T-hIL-2 at acidic pH could stimulate the proliferation of CTLL-2 cells. No IL-2 activity was present in the cell supernatant. No stimulation was observed if the lymphoma cells were treated with T-hIL-2 at neutral pH or with hIL-2 at acidic pH. This result show that the T domain may be used for the anchoring of immunostimulant proteins to the surface of cells, and that these proteins can be detected by other cells harboring the corresponding receptor. We have also demonstrated the linkage of murine IL-3 and antibodies to cells using our T domain fusion technology. Moreover, the T domain functions similarly when the soluble protein is fused to its N- or C-terminus, allowing orientation of that protein with respect to the cell surface. Many works have shown that efficient antitumor cellular response may be obtained by gene transfer and expression of immunostimulant molecules in cancer cells. We propose our new approach as an alternative to gene transfer. It allows treatment of live, irradiated or mitomycin-treated cells as well, and avoids the use of retroviral vectors and selection of transfected cells.
Moreover, bound proteins may be studied in parallel on model membranes as well as on cells, without complex reconstitution steps.